



IDENTIFICATION OF MEDICINAL ACTIVE COMPOUNDS AND QUANTIFICATION OF STRYCHNINE AND BRUCINE IN HYDRO ETHANOLIC EXTRACT OF STRYCHNOS NUX-VOMICA LEAVES

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ABSTRACT

In this study, we have analyzed the phytochemical composition of this plant, Purity and concentration of strychnine and brucine are determined. The identification of medicinal active compounds was done with GC-MS with NIST Library. Twenty one medicinal bio active compounds were identified from the Strychnos Nux-Vomica leaf ethanolic extract. Strychnine is showing 28.43% purity and brucine was not detected in GCMS analysis. Quantified the concentration of strychnine (0.6 mg in 500mg of extract) and brucine (1.6 mg in 500mg of extract) was done by HPLC against Strychnine and Brucine standard. These compounds are having natural properties of Anti-inflammatory, Hypocholesterole, Cancer preventive, Hepatoprotective, Antimicrobial, Antioxidant, Cardio protective, Antiaging, Antialzheimeran, Antidermatitic, Immunostimulant, Anthepatotoxic, biosynthesis of steroid hormones, Nematicide, Antiandrogenic, 5-alpha reductase inhibitor, antipsychotic, analgesic, apoptotic effect, antidepressant, antidote for snake poisoning and diabetic activity. From our observations, it was very clear that, this plant is a rich source of bioactive compounds.

INTRODUCTION:

The plants retain numerous pharmacological actions such as an antimicrobial, fungistatic and to reduce stress [1, 2]. The phytochemical studies and analysis statement shows that the medicinal plant main compounds are bioflavonoids, alkaloids (naturally occurring chemical compounds), poly hydroxy phenols, amphipathic glycosides and steroids (family of polycyclic chemical compounds) [3]. The Plants yield many secondary metabolites (organic compounds that are not directly involved in the normal growth, development,

or reproduction of an organism), these alkaloids purpose as a actual chief class of protective compounds in human life. Plant compounds have biological activity that used in medications. Alkaloids are identical beneficial therapeutic compounds since of their natural actions [4, 5]. Strychnos Nux-vomica shown the occurrence of carbohydrate, alkaloid, tannin, steroid, triterpenoid and glycoside in the extract [6]. Strychnos Nux-vomica of the natural components of the plant is useful in the innovation of the therapeutic agent as well as

commercial ingredients like oil and gums. The most significant bioactive components of the plants are alkaloids, tannins, flavonoids and phenolic compounds. In India a huge number of plant species had selected for their pharmacological properties. Medicinal plants are of interest to the field of biotechnology, medicine industries depend for the production of therapeutic combinations [7]. *Strychnos nux-vomica* is a therapeutic plant distributed in India, Srilanka, Southeast Asia and Northern America. Usually seeds of *S. nux-vomica* are used for therapeutic ailments in antitumor, antimicrobial, ant convulsion, anti-amnesic and immunomodulatory effects [8]. Therapeutic and medicinal plants are widely used in the world for the treatment human illnesses from the earliest historical. In India approximately 47,000 herbal plants are identified in various regions, almost 8,000 kinds of plants have curative significant. Roughly 2,500 plants are identified in Indian for several medical applications [9]. Now a day's medicinal plants are becoming extinct. In olden days plant materials are occurred from natural sources. India is having good number of therapeutic plants through which people are getting rid of their illness [10]. In Ayurveda *Strychnos Nux vomica* Linn is used in the treatment of several sicknesses and disorders [11, 12]. It is used for curing chicken pox fever and also used for snake bite [13].

Experimental

Plant Collection & authentication of Plant

Leaves of *Strychnos Nux-vomica* were used for investigation obtained from Nellore district, Andhra Pradesh, India. The plant was authenticated by Botanical survey of India, Coimbatore. Authentication number of plant is BSI/SRC/5/23/2013-14/Tech/682.

Extraction Procedure and preparation for GCMS and HPLC

Leaves of *Strychnos Nux-vomica* is washed with distilled water, shade dried, powdered for the solvent extraction process. The crude extract was obtained by extracting 50 grams of dried plant powder in 200ml of 50% Ethanol in a water shaker for 72 hrs.

Repeatedly solvent extraction was done with the same solvent till colour less solvent obtained. The hydro ethanolic plant extract was further concentrated by using Rota evaporator at 45-50°C. After concentration, the residue occurred was dissolved in methanol and analysis is carried out by using GC-MS, 500mg of plant extract taken into a 10ml of volumetric flask, dissolved with methanol and used for HPLC analysis.

Mobile phase and Standard Preparation for HPLC

Composition of Mobile phase is used as Methanol, water and diethyl amine (55:45:0.2v/v) for HPLC. 10mg of strychnine and 10 mg of brucine taken into a 10ml of volumetric flask and dissolved with methanol and filtered with 0.22 micron filter.

Instrument, Column and Method of Gas Chromatography- Mass Spectrometry

Identification of medicinal active compounds was done by using Gas Chromatography- Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70electron volts. A Restek-5MS column (30meters x 0.25milli meter x 0.25micro meter) was used, Helium flow rate was kept 1mL/min for analysis.

Instrument, Column and Method of HPLC

Quantification of Strychnine and Brucine was done by using HPLC. Agilent HPLC was used for analysis, model-1100 with Chemstation software. A Phenomenex-ODS column (250mm x 46mm x 5micro meter) was used, the Column flow rate was kept at 1mL/min, Detector at 260nm, Run time 15 minutes for analysis.

Formula for HPLC Calculation

The same formula used for quantification used for strychnine and brucine [14].

$$\%c \text{ Estimation} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times 100$$

At = Area count for sample solution.
 As = Area count for standard solution.
 Ds = Dilution factor for sample.
 Dt = Dilution factor for standard.
 Ws = Weight of standard (mg)
 Wt = Weight of sample (mg)

Instrument, Column and Method of Gas Chromatography- Mass Spectrometry

Identification of medicinal active compounds was done by using Gas Chromatography- Mass Spectrometry. Shimadzu GC-MS was used for analysis, model-QP2010 and operating in Electron Impact Ionization mode at 70electron volts. A Restek-5MS column (30meters x 0.25milli meter x 0.25µmicro meter) was used; Helium flow rate was kept 1mL/min for analysis. The oven thermal program was initially kept at 50°C and hold for 5 min, rise 10°C per minute up to 150°C and hold for 8 min, rise 8°C per minute up to 250°C and hold for 2 min, rise 10°C per minute upto 280°C and hold for 29.5 min. Injector temperature was kept 280°C and injection volume was 2 µL. The EI Source temperature kept was 250°C, Quadrupole temperature kept was 150°C, Interface Temperature kept was 250°C, full scan mode, Scan range 40-600 m/z and solvent delay kept was 3 minutes.

Identification of components:

Identification of mass spectra of GC-MS was done by using the National Institute of Standards and Technology (NIST) library. Unknown components of the mass spectrum were compared with to the NIST library components and identified. The name, mass and structure of the compounds of the test sample were determined.

RESULTS AND DISCUSSION

In the present study these biological compounds were found with GCMS analysis of Strychnos nux-Vomica. These compounds are Megastigmatrienone, 1,3,4,5-

Tetrahydroxy-Cyclo hexane carboxylic acid, 3-O-Methyl-d-glucose, Hexadecanoic acid methyl ester, Cholest-5-EN-3-OL (3.Beta.), n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid methyl ester, 2-Hexadecen-1-OL, 1,4-Dioxaspiro[4.14]nonadecane, Hexadecanoic acid, 2,6,10,14,18,22-Tetracosahexaene, γ-Tocopherol, Vitamin E, Ergost-5-EN-3-OL, Strychnidin-10-ONE, Stigmast-5-EN-3-OL and Methyl Commate D (These are mentioned in **Figure-1** for GCMS chromatogram, **Table-1** for List of the Compounds and Table-2 for Structure of the compounds). Strychnine and brucine content was quantified in HPLC, these are 0.6 and 1.6 mg respectively in the 500mg of the extract. (These are mentioned in **Figure-2** for HPLC Standard chromatogram and **Figure-3** for HPLC Sample chromatogram). NIST library compounds spectrums mentioned from **Figure-4 to 23**.

The therapeutic properties of the significant component specify the veracity of the use of this medication. Megastigmatrienone is used as an Aroma compound and 3-O-Methyl-d-glucose is having Preservative properties. Hexadecanoic acid methyl ester is used in Antioxidant, Flavor, Hypo cholesterolemic Pesticide and 5-Alpha reductase inhibitor treatments. 9, 12, 15-Octadecatrienoic acid methyl ester are reports in Anti-inflammatory, Hypocholesterole, Cancer preventive and Hepatoprotective. 2-Hexadecen-1-OL showed the Antimicrobial, Anticancer and Anti-inflammatory activity. 2, 6, 10, 14, 18, 22-Tetracosahexaene is also having the Antibacterial and Antioxidant properties. γ-Tocopherol is a vitamin and used in the treatment for Anticancer, Antioxidant, Antitumor, Anti-inflammatory, Hypocholesterolemia and Cardioprotective sickness. Vitamin E is known compound used in Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, hypocholesterolemia and Immunostimulant. Ergost-5-EN-3-OL is an Antioxidant and also having hypocholesterolemia activity.

Figure-1 GC-MS chromatograms shows the Retention Time of the compounds of *Strychnos Nux-vomica*

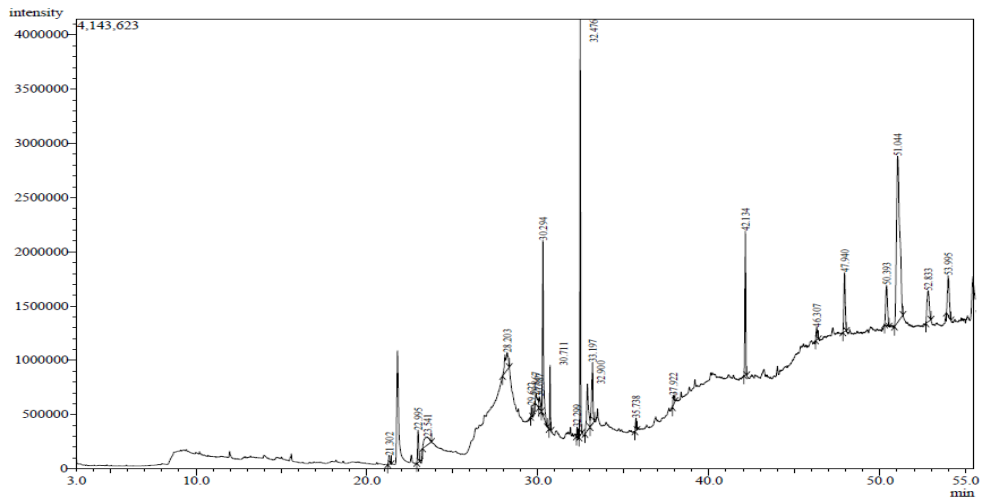
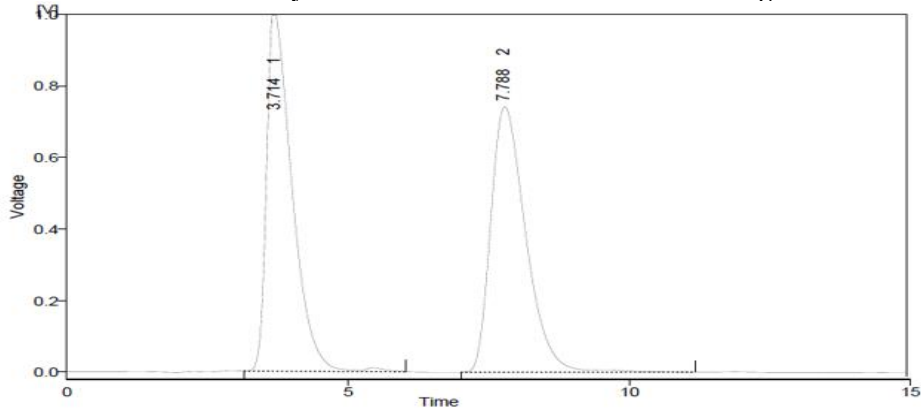


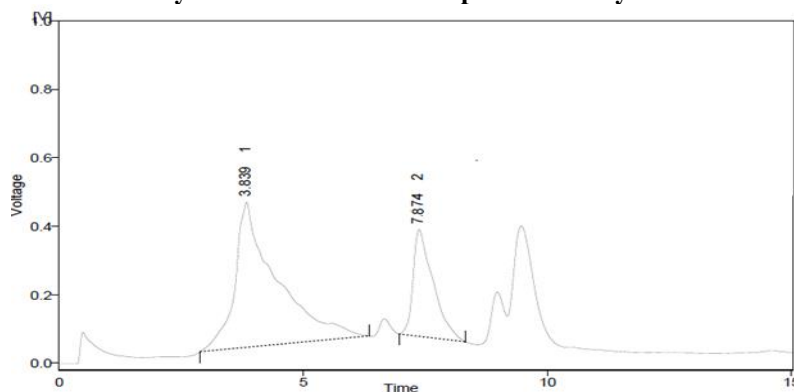
Figure 2: Retention Time of Strychnine and brucine standards chromatograms shows in HPLC



Result Table (Uncal - STD_NUXVOMICA_11.03.15)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.713	31712.159	994.058	49.6	57.3	0.48
2	7.787	32174.177	741.853	50.4	42.7	0.66
	Total	63886.336	1735.911	100.0	100.0	

Figure 3: Retention Time of Strychnine and brucine compounds in *Strychnos Nux-vomica* of HPLC



Result Table (Uncal - SAMPLE_NUXVOMICA_11.03.15)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.838	25161.659	423.541	73.8	57.6	0.75
2	7.873	8946.179	311.664	26.2	42.4	0.44
	Total	34107.838	735.205	100.0	100.0	

Table 1. List of the Compounds present in *Strychnos Nux-vomica* extract of the GC MS analysis

Sl. No	Retention Time in minutes	Compound	Molecular Formula	Molecular Weight	Area
					Area %
1	21.302	Megastigmatrienone	C ₁₃ H ₁₈ O	190	415108 0.58
2	22.995	Megastigmatrienone	C ₁₃ H ₁₈ O	190	1403906 1.98
3	23.541	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid	C ₇ H ₁₂ O ₆	192	1680694 2.37
4	28.203	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	2220057 3.13
5	29.662	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	281869 0.40
6	29.867	Cholest-5-EN-3-OL (3.Beta.)	C ₂₇ H ₄₆ O	386	88151 1.24
7	30.087	Cholest-5-EN-3-OL (3.Beta.)	C ₂₇ H ₄₆ O	386	630306 0.89
8	30.294	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	6887181 9.70
9	30.711	Hexadecanoic Acid, Ethyl ester	C ₁₈ H ₃₆ O ₂	284	1496754 2.11
10	32.299	9,12,15-Octadecatrienoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	262921 0.37
11	32.476	2-Hexadecen-1-OL	C ₂₀ H ₄₀ O	296	10492917 14.78
12	32.900	Cyclopropaneoctanoic acid	C ₂₂ H ₃₈ O ₂	334	3246047 4.57
13	33.197	9,12,15-Octadecatrienoic acid, ethyl ester	C ₂₀ H ₃₄ O ₂	306	3298198 4.65
14	35.738	1,4-Dioxaspiro[4.14] nonadecane	C ₁₇ H ₃₂ O ₂	268	418787 0.59
15	37.922	Hexadecanoic acid	C ₁₉ H ₃₈ O ₄	330	477387 0.67
16	42.134	2,6,10,14,18,22-Tetracosahexaene	C ₃₀ H ₅₀	410	4974663 7.01
17	46.307	γ-Tocopherol	C ₂₈ H ₄₈ O ₂	416	557217 0.78
18	47.940	Vitamin E	C ₂₉ H ₅₀ O ₂	430	3038192 4.28
19	50.393	Ergost-5-EN-3-OL	C ₂₈ H ₄₈ O	400	2683723 3.78
20	51.044	Strychnidin-10-ONE (Strychnine)	C ₂₁ H ₂₂ N ₂ O ₂	334	20184057 28.43
21	52.833	Stigmast-5-EN-3-OL, (3.Beta.)-	C ₂₉ H ₅₀ O	414	2688057 3.79
22	53.995	Methyl Commate D	C ₃₁ H ₅₀ O ₄	486	2769166 3.90

List of the NIST library Compounds present in *Strychnos Nux-vomica* leaves extract of the GC MS analysis

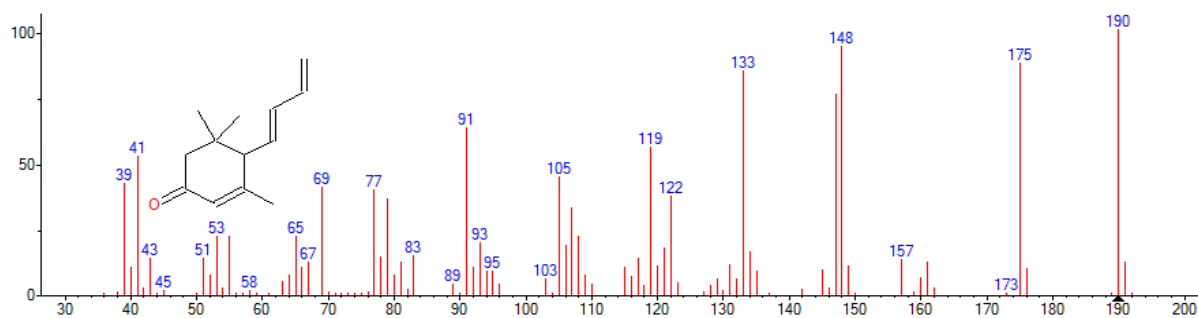


Figure 4. Megastigmatrienone

Formula: $C_{13}H_{18}O$, MW: 190, Exact Mass: 190.135765, NIST#: 47233 ID#: 3584

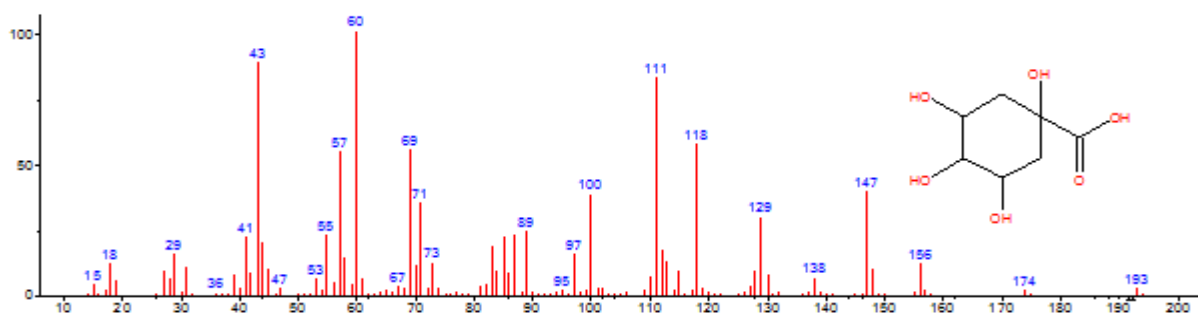


Figure 5. 1, 3, 4, 5-Tetrahydroxy-Cyclohexanecarboxylic acid

Formula: $C_7H_{12}O_6$, MW: 192, Exact Mass: 192.063388, NIST#: 229684 ID#: 28354

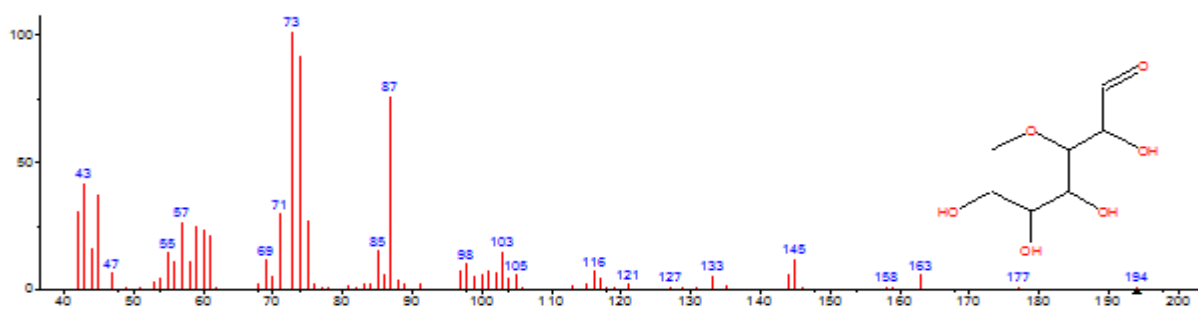


Figure 6. 3-O-Methyl-d-glucose

Formula: $C_7H_{14}O_6$, MW: 194, Exact Mass: 194.079039, NIST#: 27259 ID#: 37586

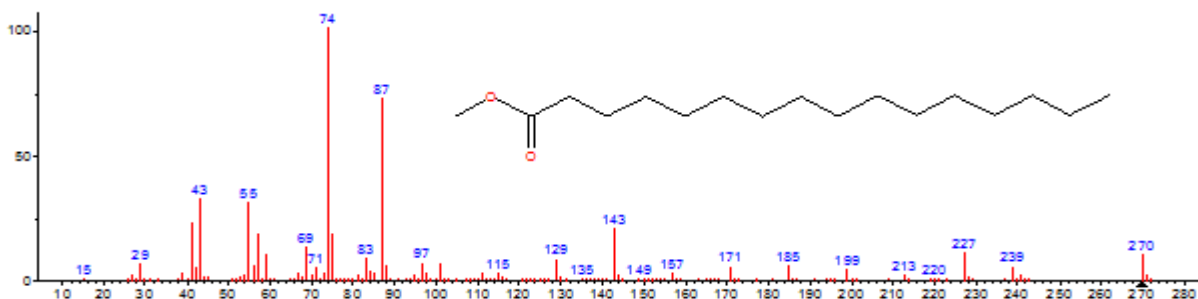


Figure 7. Hexadecanoic acid, methyl ester

Formula: $C_{17}H_{34}O_2$, MW: 270, Exact Mass: 270.25588, NIST#: 333716 ID#: 40690

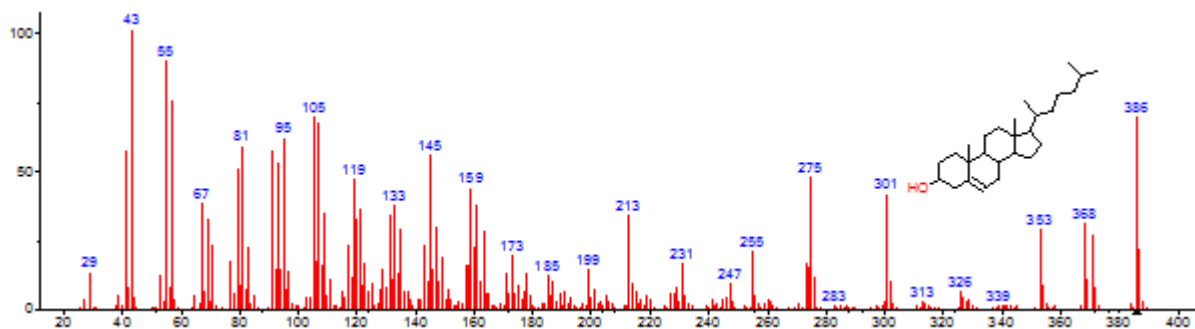


Figure 8. Cholest-5-EN-3-OL (3.Beta.)(Cholesterol)
Formula: $C_{27}H_{46}O$, MW: 386, Exact Mass: 386.354866, NIST#: 332884, ID#: 6840

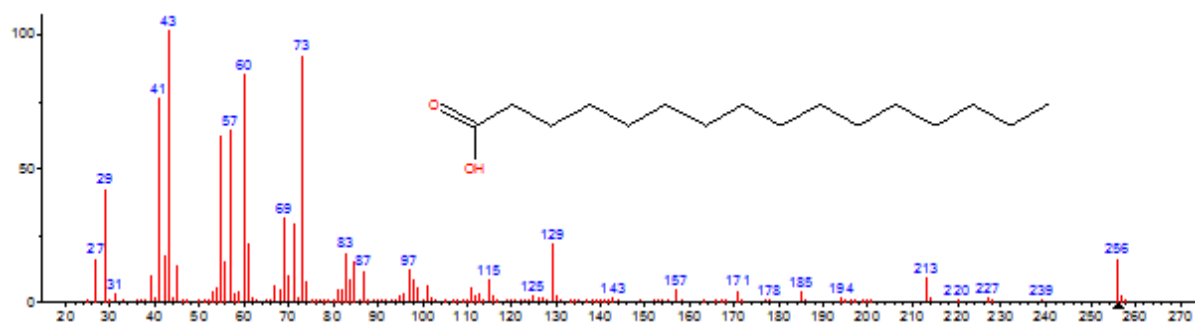


Figure 9. n- Hexadecanoic acid
Formula: $C_{16}H_{32}O_2$, MW: 256, Exact Mass: 256.24023, NIST#: 151973, ID#: 8689

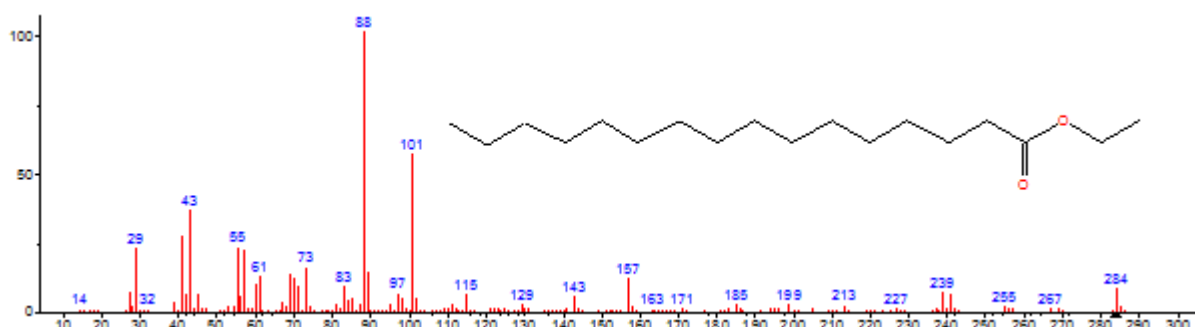


Figure 10. Hexadecanoic Acid, Ethyl ester
Formula: $C_{18}H_{36}O_2$, MW: 284, Exact Mass: 284.27153, NIST#: 233204, ID#: 52733

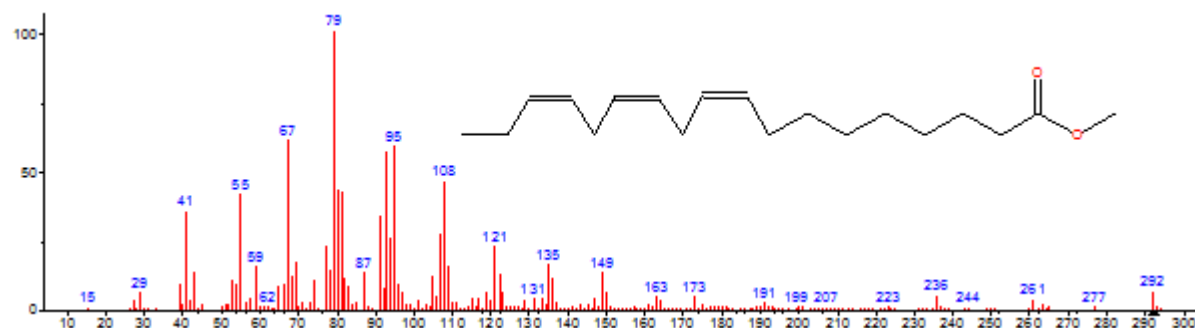
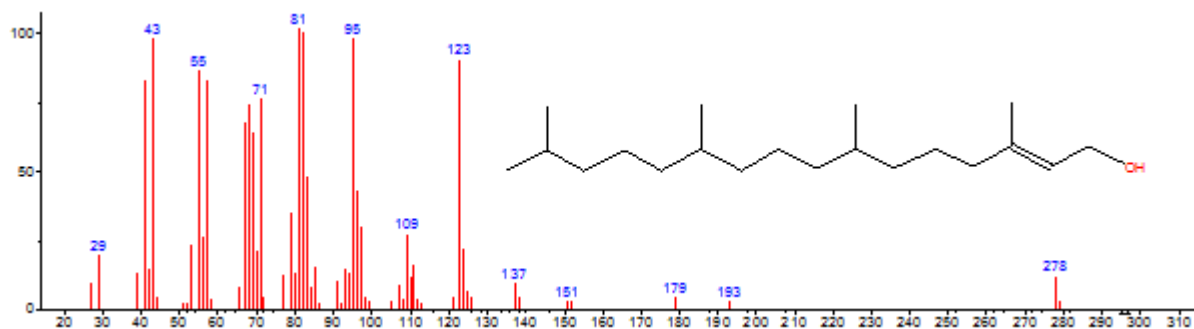
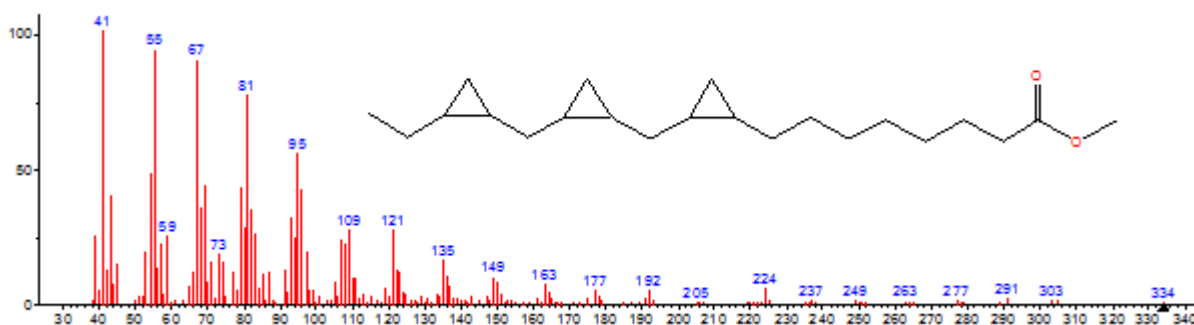


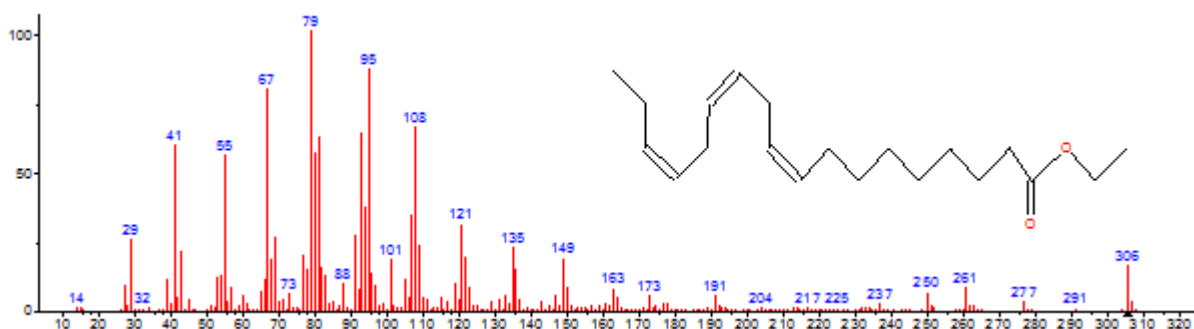
Figure 11. 9, 12, 15-Octadecatrienoic acid, methyl ester
Formula: $C_{19}H_{32}O_2$, MW: 292, Exact Mass: 292.24023, NIST#:333199, ID#: 44319



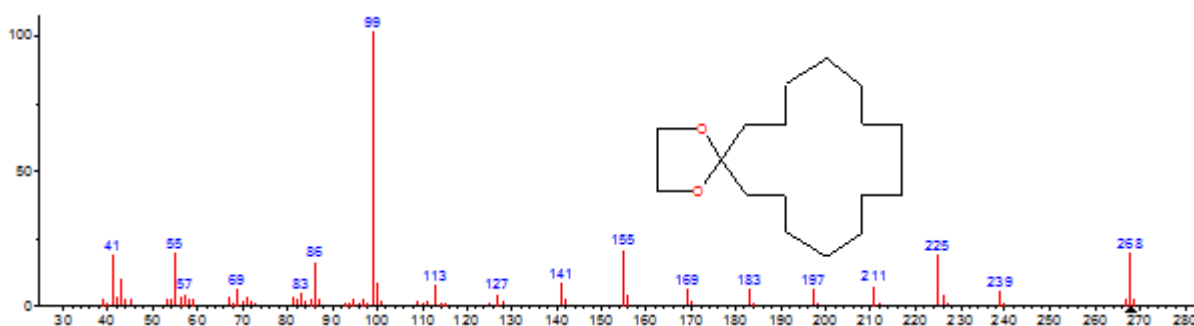
Formula: $C_{20}H_{40}O$, MW: 296, Exact Mass: 296.307917, NIST#:114703, ID#: 45842



Formula: $C_{22}H_{38}O_2$, MW: 334, Exact Mass: 334.28718, NIST#: 35867, ID#: 2585



Formula: $C_{20}H_{34}O_2$, MW: 306, Exact Mass: 306.25588, NIST#: 235982, ID#: 44703



Formula: $C_{17}H_{32}O_2$, MW: 268, Exact Mass: 268.24023, NIST#:141676, ID#: 65930

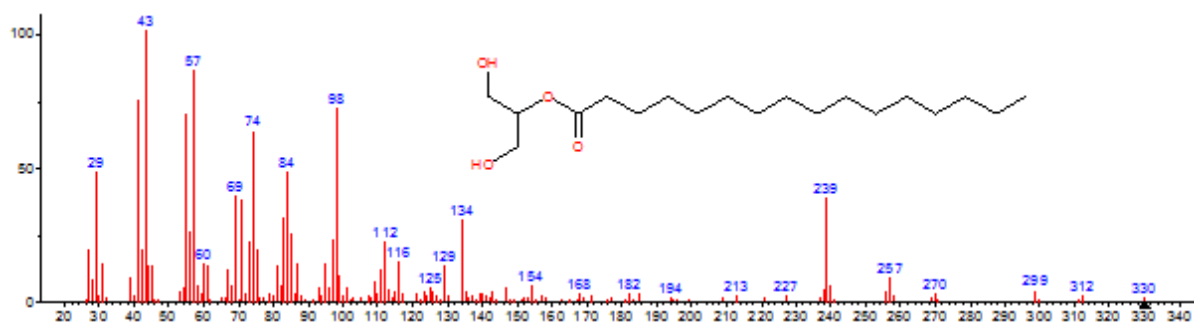


Figure 16. Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester
Formula: $C_{19}H_{38}O_4$, MW: 330, Exact Mass: 330.27701, NIST#: 15400, ID#: 7272

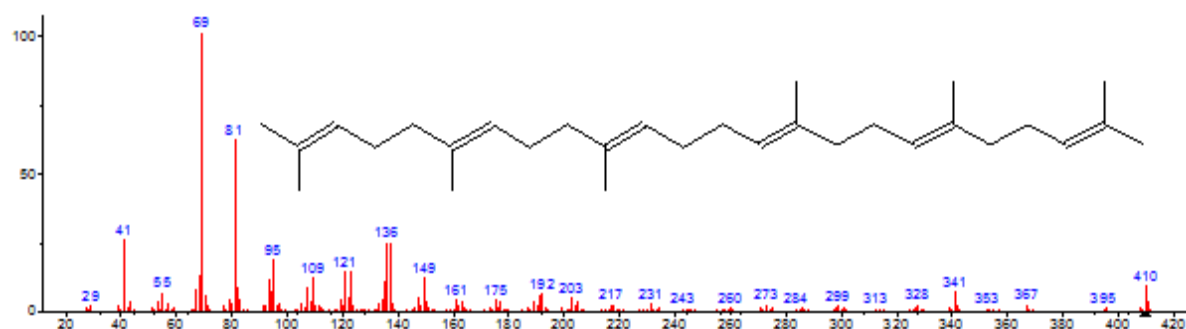


Figure 17. 2, 6, 10, 14, 18, 22-Tetracosahexaene (Supraene)
Formula: $C_{30}H_{50}$, MW: 410, Exact Mass: 410.391253, NIST#:227620, ID#: 32435

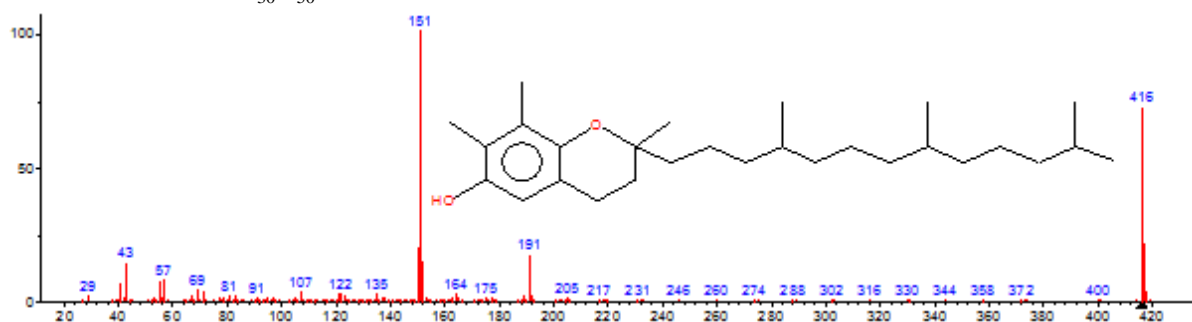


Figure 18. γ -Tocopherol
Formula: $C_{28}H_{48}O_2$, MW: 416, Exact Mass: 416.36543, NIST#: 374719, ID#: 125688

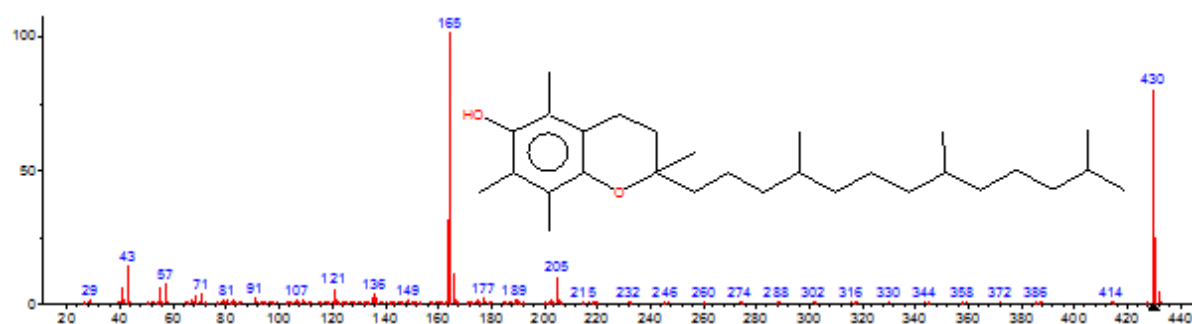


Figure 19. Vitamin E
Formula: $C_{29}H_{50}O_2$, MW: 430, Exact Mass: 430.38108, NIST#: 374713, ID#: 136995

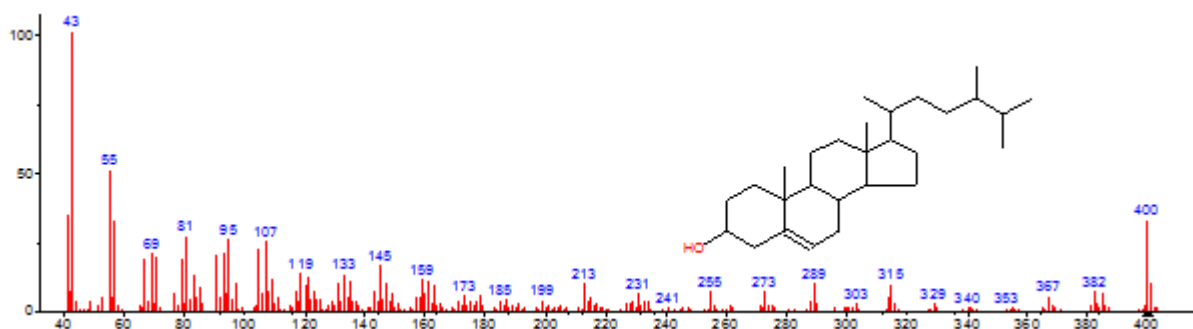


Figure 20. Ergost-5-EN-3-OL(5-Cholestene-3-ol, 24-methyl-)
Formula: $C_{28}H_{48}O$, MW: 400, Exact Mass: 400.370516, NIST#: 214174, ID#: 6688

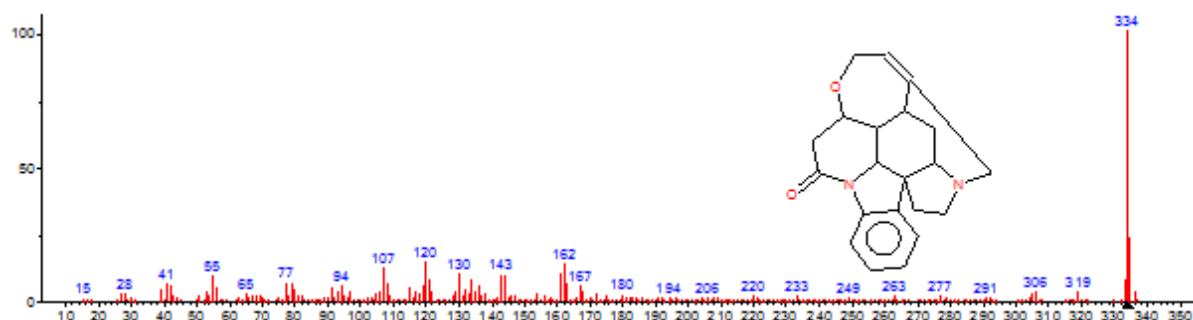


Figure 21. Strychnidin-10-ONE (Strychnine)
Formula: $C_{21}H_{22}N_2O_2$, MW: 334, Exact Mass: 334.168127, NIST#: 232994, ID#: 203578

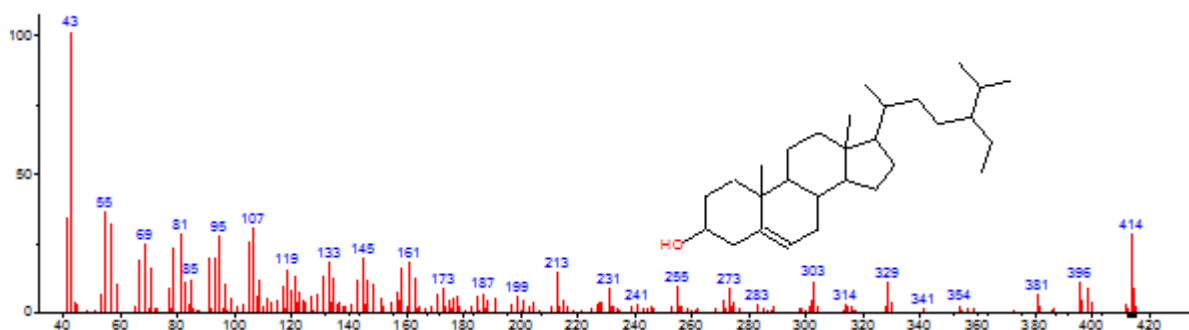


Figure 22. Stigmast-5-EN-3-OL, (3.Beta.)-(3.Beta.)- (β -Sitosterol)
Formula: $C_{29}H_{50}O$ MW: 414, Exact Mass: 414.386166, NIST#: 251915, ID#: 6717

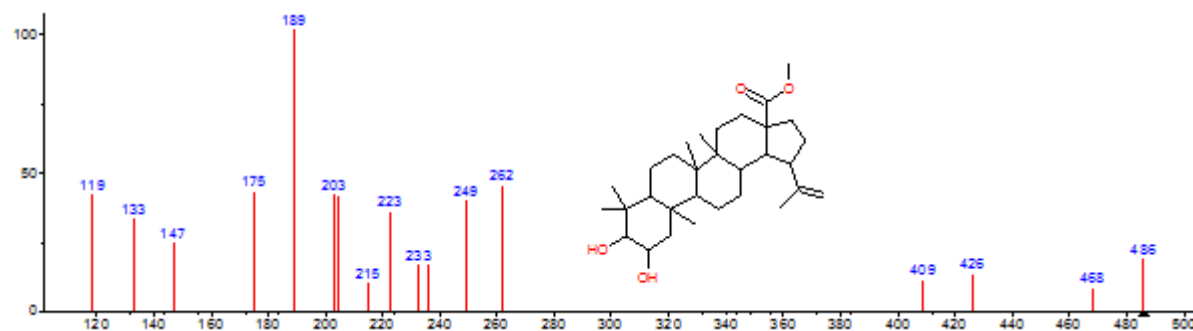


Figure 23. Methyl Commate D (Lup-20(29)-en-28-oic acid, 2, 3-dihydroxy-, methyl ester)
Formula: $C_{31}H_{50}O_4$ MW: 486, Exact Mass: 486.37091, NIST#: 64982, ID#: 155663

Stigmast -5 – EN – 3 - OL used in Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive and hypocholesterolemia illnesses. Methyl Commate D is compound used in antimicrobial, anti-inflammatory diseases [15]. Cholest-5-EN-3-OL (3.Beta.) is essential for the biosynthesis of steroid hormones, bile acids, and vitamin D [16]. n-Hexadecanoic acid is having Antioxidant, hypocholesterolemia, Nematicide, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor and antipsychotic properties [17 and 18]. Strychnidin-10-ONE is biologically active compound used in various treatments that is analgesic [19], apoptotic effect and antidepressant [20], antidote for snake poisoning [21], antitumor [22] and diabetic activity [23].

5. CONCLUSION

Bio active compounds in *Strychnos Nux-Vomica* leaves were identified by using GCMS with NIST library and Strychnine and Brucine was quantified with HPLC. These naturally occurring compounds plays crucial role in pharmaceutical and biological industries for new drug development for various diseases.

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